



IN VIVO

The Publication of the Metropolitan Association of College and University Biologists

Fall, 2003

Volume 25, Issue 1

36th Annual Fall MACUB Conference

Saturday, November 1, 2003

Wagner College

Staten Island, New York

Conference Theme: *“When Science Becomes Controversial:
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Molecular Evidence Supporting the Asian Origin Hypothesis of *Arabidopsis*

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ABSTRACT

In 1965 Bertha Berger introduced the “Asian Origin Hypothesis” of *Arabidopsis* which states that the *Arabidopsis* species originated geographically in central Asia. Berger’s supporting evidence for her hypothesis was the proliferation in Asia of many varied *Arabidopsis* species. We have revisited this hypothesis using molecular phylogenetic techniques to accurately gauge which *Arabidopsis thaliana* ecotypes lie closest to the species’ geographic origin. Thirty-one populations of *A. thaliana* were studied for phylogenetic relationships using polymorphic DNA analysis. Experiments were performed employing 18 microsatellite loci in 10 individual plants in each of the world-wide populations. Microsatellite loci were amplified by a polymerase chain reaction, electrophoresed, sized, and the polymorphisms analyzed. We found our results to be supportive of the Berger hypothesis. The most phylogenetically distant populations from the Euro-American ecotypes were those from Asia: Karagandy, Hiroshima, Pakistan, Kazakstan, Kyoto, Sakata and Tsu. These results are consistent with the original evolution of *Arabidopsis* in Asia.

INTRODUCTION

The plant species *Arabidopsis thaliana* has become the scientific community’s most prevalent model for the study of physiology, genetics, and development of higher plants. *Arabidopsis* is popular for its short growth and seed-bearing cycle (~6 wk), small size (dozens can be grown in a pot), low chromosome number (n=5), small genome size (~120 Mb), almost non-repetitive DNA, and in-depth genetic characterization.¹ Additionally, the species is the first higher plant to have its genome entirely sequenced.² *Arabidopsis thaliana* is a member of the *Brassicaceae* family. It grows wild as a winter annual in most temperate zones of the world. Ecological strains (ecotypes) have been studied with plant material collected from North America, Europe, Asia, Australia, and Africa.

Recent intensive studies into the physiology of *Arabidopsis thaliana* have brought much new knowledge to plant biology, but limited research has been conducted into the phylogeny and evolution of the plant.^{3, 4, 5, 6, 7, 8, 9}

The geographic evolutionary origins of *A. thaliana* are still obscure. It was proposed by Berger¹⁰ that the genus finds its geographic origins in central Asia. This hypothesis was based on the diverse speciation of *Arabidopsis* taxa observed by Berger while on site in central Asia. Except for Berger’s inferences, there is little experimental data supporting her “Asian Origin” hypothesis. In fact, O’Kane and Al-Shehbaz¹¹ suggested *all* species of *Arabidopsis* are European, and even hypothesized that the Himalayan and Asian *Arabidopsis* species actually belong to other genera.

Because *Arabidopsis thaliana* has become such a popular system of study, plant biologists need a better understanding of the relationships between the various populations of the plant throughout the world and their origins. There are presently many populations available for study in stock centers in Asia and North America, and researchers are sometimes at a loss to clarify why one ecotype is "better" for a particular investigation than another. Populations are often treated for convenience's sake by researchers as being quite different or similar based solely on the geographic origin of collection. Little is actually known about how far evolution has advanced among diverse *A. thaliana* populations worldwide.

Bell and Ecker¹² assigned 30 microsatellite loci to the linkage map of *A. thaliana*. In order to address Berger's hypothesis using molecular methods, we have surveyed DNA microsatellite variation in 31 *A. thaliana* populations scattered throughout the world on five continents, including ten Asian ecotypes (Table 1).

MATERIALS AND METHODS

Plants And Plant Growth

The Tanzania ecotype was donated by Roy Gereau (Missouri Botanical Garden, St. Louis, USA). New Zealand seeds were donated by Mary Skotnicki (Australian National University). Canary Islands, Cape Verde Islands and LaPal ecotypes were obtained from Nobuharu Goto (Miyagi University of Education, Sendai, Japan). All other ecotypes were obtained from Randy Scholl (Ohio State University, Columbus, Ohio, USA). All seedlings were germinated under sterile conditions and grown under the methods previously described in Vander Zwan et al.⁹

DNA Extraction

Ten individual plants from each of the 31 populations were examined for the variation in microsatellite polymorphism among populations for a total of ~310 plants. The DNA was extracted from whole seedlings that typically weighed 0.1-0.7 gm. The CTAB method from Keller¹³ was used for DNA extraction. Plant tissue was frozen overnight at -70°C and homogenized in an ice-cooled mortar and pestle.

Polymerase Chain Reaction and Analysis

Eighteen PCR Simple Sequence Length Polymorphism (SSLP) primer sets (Table 2)¹² were purchased from Research Genetics, Inc. and used to amplify microsatellite loci from plant samples. These polymorphic loci are DNA sites that have been successfully used in many eucaryotic systems to reconstruct phylogenetic relationships.^{14, 15, 16, 17, 18} The PCR amplification conditions principally followed the directions of Bell and Ecker.¹² Microsatellites were amplified in 20 µl reactions containing: 10-20 ng plant DNA, 10% ThermoPol buffer (New England Biolabs, Inc.), 5 pmoles forward and reverse primers, 200 µM dNTPs (New England Biolabs, Inc.), and 0.5-1.0 unit Taq polymerase. All amplification was performed in a Mastercycler Gradient Thermocycler (Eppendorf, Inc.). The PCR products were electrophoresed on 3-4% high-resolution agarose gels (Metaphor Agarose, FMC Bioproducts). Metaphor agarose from FMC has been shown to resolve DNA fragments down to a difference of ~4 basepairs.¹⁹ The products on the agarose gels were stained with ethidium bromide and imaged using an Ultralum gel documentation system (Ultralum, Inc.) and Scion computer software (Scion, Inc.). Molecular weights were analyzed and calculated with Collage Version 4.0 (Image Dynamics Corporation). The raw DNA allele lengths were used in phylogenetic

Table 1. *Arabidopsis* ecotypes used in this study. The ten ecotypes in bold are from Asia.

Designation	Name	Geographic Origin	Donated by
Abd	Aberdeen	Scotland	Randy Scholl
Bay-0	Bayreuth	Germany	Randy Scholl
Berk	Berkeley	USA	Randy Scholl
Bor	Bor	Algeria	Nobuharu Goto
Br-0	Bruun	Czech Republic	Randy Scholl
Can	Canary Islands	Canary Islands	Nobuharu Goto
Con	Condara	Tajikistan	Randy Scholl
Ct-1	Catania	Italy	Randy Scholl
Cvi	Cape Verde Islands	Cape Verde Islands	Nobuharu Goto
Esp	Espoo	Finland	Randy Scholl
Hiro	Hiroshima	Japan	Nobuharu Goto
Ita-0	Ita	Morocco	Randy Scholl
Kara	Karagandy	Kazakstan	Nobuharu Goto
Kas-1	Kashmir	India	Randy Scholl
Kaz2	Kazakstan	Kazakstan	Randy Scholl
Kin-0	Kindleville	USA	Randy Scholl
Kyot	Kyoto	Japan	Nobuharu Goto
Lapal	Lapal	Cape Verde Islands	Nobuharu Goto
Lc-0	Loch Ness	Scotland	Randy Scholl
Ler	Landsberg Erecta	Germany	Randy Scholl
Lip-0	Liowiec	Poland	Randy Scholl
Mt-0	Martuba	Libya	Randy Scholl
Mv-0	Martha's Vineyard	USA	Randy Scholl
Nz-2	New Zealand	New Zealand	Mary Skotnicki
Paki	Pakistan	Pakistan	Nobuharu Goto
Pami	Pamiro-Alay	Tajikistan	Nobuharu Goto
Pogo	Point Gray	Canada	Randy Scholl
Saka	Sakata	Japan	Nobuharu Goto
Tanz	Tanzania	Tanzania	Roy Gereau
Tsu	Tsu	Japan	Randy Scholl
Yo-0	Yosemite	USA	Randy Scholl

analyses of the *Arabidopsis thaliana* populations. Distance Calculations

Phylogenetic relationships were ascertained among the various ecotypes using several computer software packages. Microsat²⁰ was used to calculate microsatellite-based genetic distances between populations: both Ddm and Da distances. Those genetic distances were then evaluated with Phylip²¹ to calculate neighbor-joining relationships between populations. The neighbor-joining data generated by Phylip was formed into a graphic tree by Treeview.²²

Genetic distances were calculated as the absolute difference between alleles (Da)²³ and the square mean difference between alleles (Ddm).^{25, 18} The Da method of measurement is not based on an evolutionary model, but on the product of allele frequencies shared between populations. The Ddm is an estimation based on the Stepwise Mutation Model of genetic change. There are tradeoffs between the two modes of calculation. Although Da does not increase linearly over time, becoming extremely flat as time becomes large, it accurately reconstructs

TABLE 2. Size of PCR products (in bp) for 18 microsatellite loci from 31 *Arabidopsis thaliana* populations. n.a.= not amplified by primer Used.

Ecotypes	ATHACS	ATHCHIB	ca72	nga8	nga32	nga59	nga151	nga158	nga162	nga168	nga172	nga225	nga707	nga1107	nga1145	Bio2	PhyC	GapAB
Abd-0	n.a.	n.a.	223	152	255	122	162	95	94	158	138	139	128	143	217	168	200	130
Bay-0	275	86	228	158	298	114	104	97	77	149	150	142	116	134	194	175	200	125
Berkeley	275	n.a.	246	120	262	122	146	109	120	151	160	121	125	134	225	162	200	122
Bor	272	n.a.	260	173	250	133	171	99	94	144	165	123	n.a.	150	200	n.a.	200	143
Br-0	266	86	232	188	247	119	140	109	90	140	154	132	121	134	217	226	200	122
Can	291	n.a.	231	119	269	120	172	101	94	138	188	n.a.	115	141	204	200	200	153
Condara	n.a.	n.a.	239	160	226	105	118	105	81	126	154	87	112	127	183	n.a.	217	139
Cvi	275	69	225	132	231	135	180	96	114	135	169	128	124	141	209	163	200	126
Ct-1	296	106	232	183	245	136	122	102	90	135	151	196	134	131	200	n.a.	200	122
Es-0	294	86	223	132	258	127	122	97	94	135	138	170	128	124	180	168	215	130
Hiro	285	119	250	201	273	110	140	170	98	140	134	n.a.	165	138	217	228	228	257
Ita-0	202	89	232	233	264	n.a.	171	93	85	135	189	99	122	131	188	175	200	139
Kara	n.a.	n.a.	235	183	255	117	120	100	n.a.	140	185	189	n.a.	147	200	170	226	147
Kas-1	294	101	238	172	267	119	134	127	88	144	140	113	125	136	203	134	200	143
Kaz2	280	76	238	170	257	160	230	100	94	141	169	185	129	129	199	175	200	128
Kin-0	288	106	241	168	255	170	148	127	99	149	142	132	112	126	206	175	234	145
Kyot	292	86	240	171	245	168	118	191	90	168	181	99	129	140	209	200	200	143
Lapal	267	n.a.	225	128	257	117	158	100	n.a.	123	173	n.a.	125	138	200	197	200	135
Lc-0	288	89	242	165	270	122	128	162	99	135	158	119	131	136	199	175	200	139
Ler-0	284	73	241	190	251	111	120	105	81	126	134	194	121	136	217	212	212	143
Lip-0	236	93	232	116	276	122	114	117	90	126	138	191	116	136	197	122	200	107
Mt-0	n.a.	n.a.	246	91	251	122	100	105	90	126	172	145	139	138	206	162	200	122
Mv-0	281	98	234	160	245	122	134	113	81	155	166	236	122	134	203	n.a.	n.a.	n.a.
Nz-2	285	74	249	192	269	130	150	100	114	132	142	123	124	131	202	155	200	125
Paki	n.a.	n.a.	240	160	300	242	120	99	90	140	180	n.a.	129	160	217	145	200	145
Pami	n.a.	n.a.	237	153	252	167	120	114	n.a.	131	165	100	125	135	200	139	223	149
Pog-0	294	74	257	174	315	151	137	113	96	149	149	121	137	149	210	175	200	139
Saka	n.a.	n.a.	200	197	248	112	111	91	98	130	159	105	138	122	119	170	224	130
Tanz	n.a.	n.a.	231	145	n.a.	120	112	100	134	146	n.a.	n.a.	125	124	200	155	200	130
Tsu-0	271	104	223	278	258	163	112	117	98	151	177	124	121	138	197	159	200	120
Yo-0	268	80	236	165	248	136	128	109	92	146	160	219	116	134	203	122	200	122

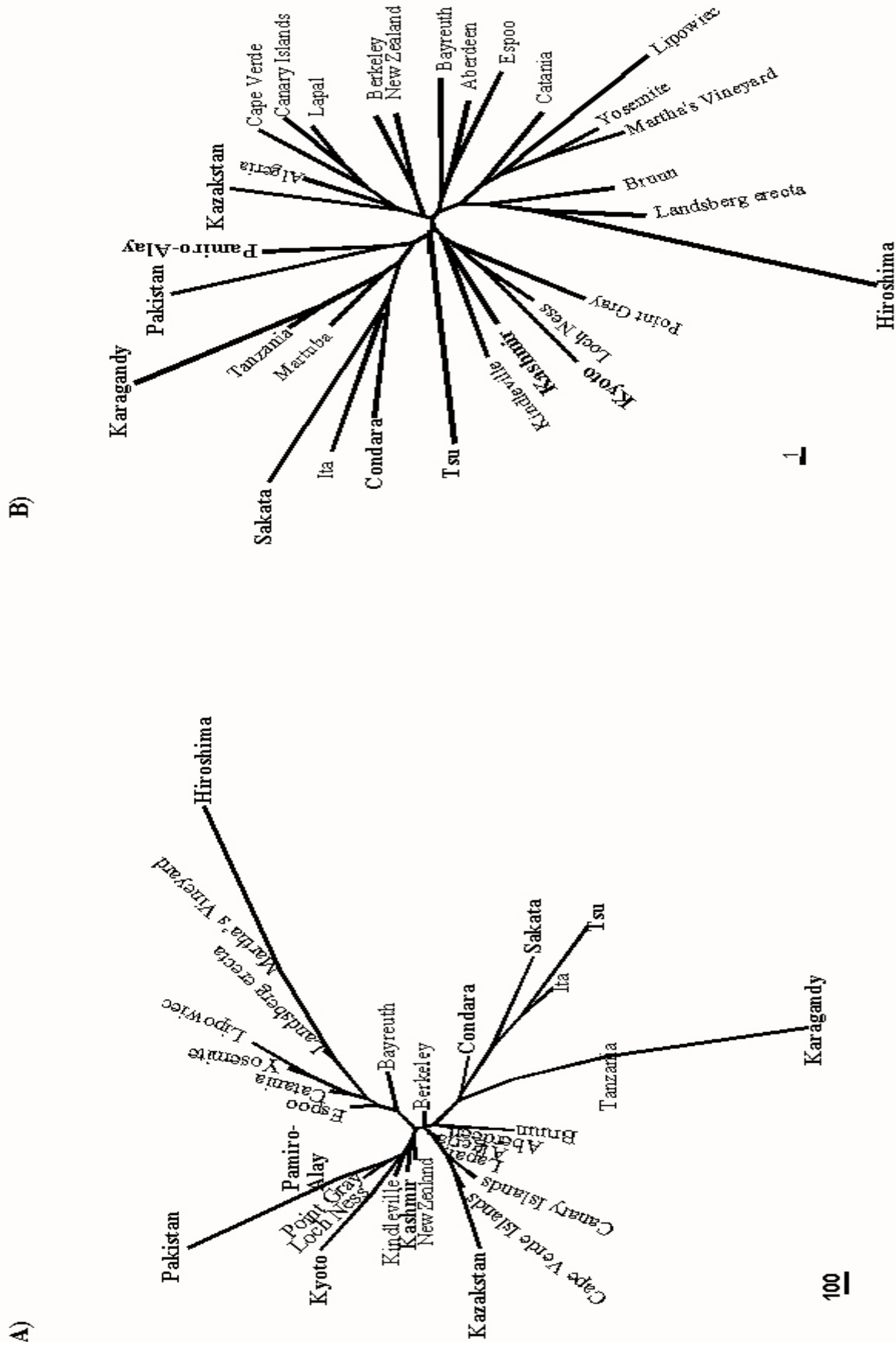


Figure 1. Unrooted radial trees of 31 populations of *A. thaliana* constructed based on the distance matrix generated by the (A) square mean difference between alleles ($\Delta \mu^2$) and (B) the absolute difference between the alleles ($\Delta \mu$). The trees were generated by the Treeview program (Page 1996). Plant populations in bold are the Asian ecotypes.

phylogenetic development over short evolutionary spans between closely-related populations.²³ The Ddm calculation is specifically designed to reduce variance, remove dependence on population sizes, and increase distance linearly over large time spans.^{24, 15, 18}

RESULTS

To examine the Berger hypothesis,¹⁰ neighbor-joining radial trees²⁵ were constructed on the basis of genetic distance obtained by analyzing microsatellite polymorphisms. Previously published data⁴ demonstrated that *Arabidopsis* microsatellite allele-length differences are due to changes in the number of DNA repeat copies in the loci.

On the basis of these polymorphic changes, distance matrices were generated by the Da and Ddm calculation methods, and unrooted, neighbor-joining trees constructed (Fig. 1). The two trees (Figs. 1A and 1B) appear similar in the relationships between the populations. The Asian populations examined consistently appeared as more distant ecotypes in both the unrooted trees that were generated. Using the Ddm analysis (Fig. 1A), the most phylogenetically distant populations were Asian in origin: Karagandy, Pakistan, Sakata, Kazakstan, Hiroshima, and Tsu. These populations were found at the ends of phylogenetic branches. The most distant populations in the Ddm tree were Karagandy and Hiroshima (Fig. 1A). The tree calculated by the Da method²³ (Fig. 1B) mirrored the Ddm tree with the Asian populations at the ends of the most distant branches (Hiroshima, Kyoto, Tsu, Condera, Sakata, Karagandy, Pakistan, Kazakstan). The overall branch-lengths in Figure 1B appeared to be shorter and more symmetrical ("bush-like") than the Ddm tree (Fig. 1A) which shows a more bifurcating, tree-like pattern. The Da analysis also placed Hiroshima and Karagandy as the most

phylogenetically distant populations (Fig. 1B).

DISCUSSION

Bowcock et al.¹⁵ demonstrated that the analysis of polymorphic microsatellite sites is useful and accurate for reconstructing the phylogeny of humans with high precision, so we chose the same genetic distance calculation (Ddm) to examine intraspecific relationships among *Arabidopsis thaliana*. We found that our neighbor-joining trees based on the Ddm (Fig. 1A) and Da (Fig. 1B) calculations were similar. The trees in both analyses demonstrated that the Asian populations either appeared at the ends of branches or on the more distant branches. This result is more evident in the Stepwise Mutation analysis (Fig. 1A). In this tree, the Asian populations (Karagandy, Tsu, Sakata, Hiroshima, Kazakstan, Kyoto, and Pakistan) form a "halo" at the ends of branches around the more central Euro-American populations. Also, the Ddm tree is in a highly branching spatial pattern that suggests a clearer ecotypic phylogeny.

The general geographic trend of both trees (Figs. 1A and 1B), from the most distant populations inward, is east to west. If the Asian Origin Hypothesis were correct, the populations separated for the longest period from the European clades would be on the most distant branches, as are all the Asian populations. Hypothetically, races geographically closest to the *A. thaliana* origin have been reproductively isolated from the other races for the longest period and would demonstrate that long isolation by large phylogenetic distances from those other populations.

Karagandy and Hiroshima are the most phylogenetically distant populations from the others (Figs. 1A and 1B), as one would predict with radiation outward from Asia. We hypothesize, based on its large phylogenetic distance from the European clades and

Berger's botanical observations^{10, 15} that the Karagandy ecotype from Kazakstan presently is the closest candidate to the geographic origin of *Arabidopsis*. Hiroshima is a less likely candidate as the origin, despite its phylogenetic distance, because it originates on the island of Japan and not on the mainland of Asia. It seems more likely that Hiroshima is descended from a central Asian population and is a "recent" immigrant to Japan from the mainland.

Findings from two other phylogenetic studies also suggest long separation between European and Asian populations. Innan et al.⁶ employed a large number of Japanese *Arabidopsis* strains in their investigation; their data showed the Japanese ecotypes (Sakata, Hiroshima, Yamagata, Fukuoka, Mie and Ishikawa) grouped together in deep branches far from European strains. The survey by Todokoro et al.⁴ revealed that a European ecotype, used as an outgroup in another study of Japanese populations, was the most distant phylogenetic population from the rest.

The results of our present study, as well as those of Innan et al.⁶ and Todokoro et al.,⁴ strongly support Berger's "Asian Origin" Hypothesis, but final conclusions will need to wait until more ecotype samples can be obtained and studied to give us a more detailed picture. In order to pinpoint the geographic origin of *Arabidopsis* and give greater resolution, we will need a greater number of Asian populations to study—especially from central Asia in the regions of Kazakstan, Tajikistan, Afghanistan, Uzbekistan and Kyrgyzstan. We will continue studies into worldwide populations so that better descriptions may be made of all the *A. thaliana* clades.

ACKNOWLEDGMENTS

The Authors wish to thank Randy Scholl, Nobuharu Goto, Mary Skotnicki and Roy

Gereau for their kind donation of seed. We also thank Lisa Campanella for her generous help in editing this article. This work was supported primarily by a Sokol grant for undergraduate research from Montclair State University.

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Parkinson's Disease

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First mentioned in 1817 by James Parkinson, M.D., Parkinson's Disease, *paralysis agitans*, also known as shaking palsy is a chronic disorder of involuntary motor control, characterized by an involuntary shaking of the limbs and rocking of the torso. The idiopathic (no known cause) type affects approximately 50,000 Americans representing less than one percent of the population¹. Parkinson's Disease is generally a disease of the elderly with in most cases the onset is recorded between 50 to 60 years of age. When it occurs in individuals forty and under it is referred to a Young Onset Parkinson's. Parkinson's like symptoms have been recorded in cases of head injury and poisoning by some illegal and legal drugs. In the earliest stages of the disease the shaking may be almost imperceptible and the rocking absent. As the disease progresses, a progressive loss of motor skills is evident and may lead to paraplegia. While the outward manifestations of the disease are the most notable symptoms, it is the induced symptoms that are the most devastating. Since this disease affects the neuromuscular system, its effects on the parasympathetic musculature can prove disastrous. Autonomic functions such as peristalsis, diaphragm activity, heart and excretory muscles may be compromised to such an extent that the patient may require assistance to perform basic life functions. In addition, medical conditions including decubitis ulcers (bed sores), urinary tract infections, impacted bowels and pneumonia may occur as a function of the secondary lifestyle forced by the loss of muscular control.

Causes, Symptoms and Diagnosis

Parkinson's disease is caused by the degeneration of the dopaminergic nigro-striatal pathways between the substantial nigra and the basal ganglia or the striatum in the brain². As a result of this degeneration, there is an 80 - 90% depletion in the available dopamine in the striatum^{3,4}. This depletion results in a loss of fine control of the normal background movement and muscle tone mediated by the extra pyramidal pathways which are in turn affected by the striatum. The earliest stages of the disease often go unnoticed as the early symptoms, fatigue and weakness, can often be mistaken as signs of aging. As the disease progresses the face loses expression and 'animation,' known as mask face and then the patient may become unusually slow. These symptoms give way to the shaking and tremors that are characteristic of the disease⁵.

While the external manifestations can be animated and at times dramatic, there are currently no known blood or laboratory tests for Parkinson's disease. Thus, diagnosis of the disease involves observation and monitoring of the patient by the clinician for the four cardinal signs of the disease, tremor, rigidity, akinesia (bradykinesia) and postural instability, or T.R.A.P., after the tremors start to be manifested.

Treatment, Cure and Prognosis

There is no known cure for Parkinson's Disease. Treatment is generally by oral administration of drugs such as:

1. Symmetrel (Amantadine) - a synthetic antiviral first introduced as an agent for prophylaxis of influenza. It was later found to cause symptomatic improvements in Parkinson's Disease. Its mechanisms are not fully understood, but it appears to facilitate dopaminergic responses in the CNS. While it is less effective than Levodopa it can be additionally beneficial to patients in whom the effectiveness of Levodopa is not optimal.

2. Parlodel (Bromocriptine) - a synthetic dopamine agonist that is chemically related to ergot alkaloids and lysergic acid. It stimulates dopamine type-2 receptors and antagonizes type-1 receptors in the hypothalamus and the neostriatum of the CNS⁶.

3. Permax (Pergolide) - a drug which directly stimulates post synaptic dopamine receptors in the nigrostriatal system. It causes less nausea and orthostatic hypotension than bromocriptine. It may be effective in patients who have become tolerant to bromocriptine.

4. Sinemet, a combination of Carbidopa and Levodopa. Levodopa is the chemical precursor to dopamine. It is converted to dopamine both in the alimentary tract and the CNS. For Levodopa to be effective it must cross the blood brain barrier before conversion to dopamine. Only about 1% of orally ingested levodopa remains bioavailable for conversion in the CNS, as dopamine can not cross the blood brain barrier. Carbidopa, a noncompetitive decarboxylase inhibitor, inhibits the peripheral conversion of Levodopa to dopamine. This increases its bioavailability in the CNS. Carbidopa does not cross the blood brain barrier, and

therefore does not inhibit Levodopa-dopamine conversion in the CNS.

5. Elderepryl (Selegiline), also known as deprenyl and deprenil, is an oral agent used in combination with Sinemet. Selegiline is a noncompetitive antagonist of monoamine oxidase type B (MAO-B). This enzyme metabolizes dopamine in the CNS rendering it ineffective as a neurotransmitter.

Parkinson's Disease is 100% fatal. While the disease does not cause death, its cumulative effects on the body eventually causes the patient to expire. In most cases the exitus is the result of pneumonia induced by the immobility of late stage Parkinson's.

Future Outlook

The future looks bright for Parkinsonian patients. With advances in genetic engineering, especially in areas such as gene therapy and stem cell research, there is hope on the horizon. These exciting fields offer the promise of regenerating or growing entirely new dopamine producing cells in the pallidum. Not so revolutionary, but highly controversial, and reportedly showing varying degrees of success are

1. Palidotomy: The neurons in the pallidum are burned out by a thin probe threaded through a small hole in the skull. This process can cripple the patient or worse prove fatal if not done properly.

2. Thalamotomy : Similar to palidotomy, it is performed on the thalamus.

3. Cell Replacement: Pallidial cells from aborted fetuses are used to replace those of patients. Performed in Sweden, this surgery has been banned in the United

States because of moral concerns. It is also not very practical as the cells from as many as twelve fetuses are needed to perform each procedure.

Conclusion

The pathologic changes of Parkinsonism are well known. The major changes involve the loss of dopamine containing neurons in the substantia nigra and other pigmented nuclei of the brain. Viral etiology and a possible connection to toxic drugs such as cyanide and agricultural pesticides have been proposed but not convincingly confirmed. Genetic influences have been suggested but not conclusively proven. Dopaminergic, anticholinergic, antihistamines and a synthetic antiviral drug, amantidine, improve most patients for 5 -10 years and beyond. These therapies along with physical and rehabilitation therapy help patients for a longer period than previously possible. One should remember however, that Parkinson's Disease is a chronic, progressive disease that eventually leads to permanent disability.

References

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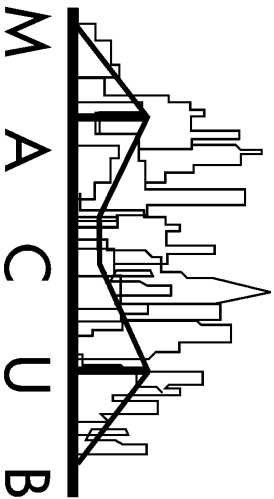
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Sample Abstract

Design and Construction of a RET Mutant Expression Vector. Eduardo Areche, and Ramona Kennedy, Montclair State University. Faculty Mentor: Dr. Quinn C. Vega.

RET is a transmembrane tyrosine kinase activated in response to a ligand. Under normal circumstances, the ligand binds to the extracellular domain leading to activation of the cytoplasmic kinase domain. In specific disease states such as multiple endocrine neoplasia (MEN) 2A and 2B, the receptor is activated in the absence of this ligand leading to thyroid tumors. MEN2A is caused by a mutation in the extracellular domain leading to receptor dimerization and activation. MEN2B is caused by a mutation in the cytoplasmic domain leading to increased activity and altered autophosphorylation. As yet, the sites of autophosphorylation in the MEN2B mutant have not been identified. In order to analyze the phosphorylation pattern and activity of the MEN2B mutant, it would be beneficial to express and purify the protein. In order to rapidly express and purify the RET mutant, a bacterial system will be used. This system consists of a plasmid containing an ampicillin resistance gene and a multi-cloning site located behind the glutathione-S-transferase (GST) gene. Using DNA amplification techniques and other molecular biology tools, this vector will allow us to make a protein consisting of GST and the kinase domain of RET. This fusion protein can then be purified using GST's affinity for glutathione. By attaching glutathione to beads, the fusion protein can be separated from the column using excess glutathione. Upon purification of the kinase, enzyme activity and function can be further analyzed.



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